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ABSTRACT

The *in vitro* activity of six synthetic peptides against 36 strains of *Chlamydia* from different origins was investigated. Clavanin MO proved to be the most active peptide, reducing the inclusion number of all *Chlamydia* strains from 8 different species tested by ≥50% at 10 µg.ml^{-1}. Mastoparan L showed an equal activity against *C. trachomatis*, *C. pneumoniae*, *C. suis* and *C. muridarum,* but did not exert any inhibitory effect against *C. psittaci*, *C. pecorum*, *C. abortus* and *C. avium* even at 80 µg.ml^{-1}. These data suggest that clavanin MO could be a promising compound in the prevention and treatment of chlamydial infections.

Running title

In vitro sensitivity of *Chlamydia* to synthetic peptides

INTRODUCTION

Chlamydiae are obligate intracellular Gram-negative bacteria belonging to the family of Chlamydiaceae, genus *Chlamydia*, including eleven species that affect a wide range of vertebral hosts, causing significant diseases both in humans and economically important domestic animals such as sheep, poultry and a variety of wildlife.[1] The three *Chlamydia* species infecting humans are represented by *C. trachomatis*, responsible for urogenital diseases and associated with neonatal conjunctivitis and pneumonitis, *C. pneumoniae*, causing respiratory infections and associated with cardiovascular diseases, and *C. psittaci*, transmitted occasionally from infected birds to humans, causing psittacosis. Chronic or persistent infections have been reported for these *Chlamydia* species. In particular, persistent *C. trachomatis* infections can cause long-term sequelae including pelvic

inflammatory disease and tubal factor infertility.[2] The *Chlamydia* species infecting other animals are represented by *C. psittaci* (in birds and mammals), *C. suis* (in pigs), *C. felis* (in cats), *C. pecorum* and *C. abortus* (in sheep, goats, cattle, pigs), *C. caviae* (in guinea pigs), *C. muridarum* (in mice), and the two recently proposed species of *C. avium* (in pigeons and parrots) and *C. gallinacea* (in chickens).[3] Chlamydiae infecting animals can cause a variety of diseases ranging from mild infections like conjunctivitis and enteritis to more serious disorders such as pneumonia, polyarthritis, pericarditis, encephalitis and reproductive problems (especially in sows and boars).[4] Although *C. psittaci* and *C. abortus* zoonotic potential is well known, the zoonotic transmission of the other species is still unclear and has to be investigated. Both human and animal chlamydial infections are primarily treated with tetracycline or its derivatives and macrolides.[5] Despite the *in vitro* antimicrobial activity of these drugs against *C. trachomatis* and most of the other *Chlamydia* species, there are reports of tetracycline treatment failure in humans.[6] In addition, a stable tetracycline-resistance associated with *tet*(C) genomic islands integrated into the chlamydial chromosome has been described for *C. suis* strains isolated from pigs,[7] as well as a tetracycline resistance expressed *in vitro* by *C. trachomatis* following co-culture with tetracycline-resistant *C. suis* strains.[8]

In this context, the formulation of synthetic peptides with antimicrobial activity has become extremely attractive. Several studies demonstrated the *in vitro* antimicrobial activity of synthetic peptides against various antibiotic-resistant Gram-positive and Gram-negative bacteria. Some studies evaluated the *in vitro* activity of natural and synthetic host defence peptides against *Chlamydia* spp., demonstrating protegrins and α-helical peptides to be the most active peptides and *C. trachomatis* the most sensitive among *Chlamydia* species tested.[9] Our recent investigation showed substantial *in vitro* activity of cathelicidin SMAP-29, in comparison with five other cathelicidins, against *C. trachomatis* and *C. pneumoniae*.[10] On the other hand, the potent SMAP-29 antimicrobial activity is associated with toxicity towards mammalian cells.

Infections caused by antibiotic-resistant bacteria such as the group called ESKAPE (*Enterococcus faecium*, *Staphilococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.) have contributed to increases in patient mortality. Due to these facts, the formulation of synthetic peptides with improved solubility and antimicrobial activity and lower toxic effects towards mammalian cells has become extremely attractive.[11] The good activity of synthetic peptides, such as the wasp-generated peptide mastoparan,[12] clavanins (cationic α-helical peptides from the marine tunicate *Styela clava*),[13] and *Pa*-MAP (an alanine-rich peptide with hydrophobic amino acid residues from the polar fish *Pleuronectes americanus*) against some Gram-positive and Gram-negative bacteria has been reported.[14,15]

Since the anti-chlamydial activity of these peptides has not been tested so far, we evaluated the *in vitro* activity of multiple synthetic peptides against *Chlamydia*. This evaluation aims to assess a possible therapeutic use in clinically unresolved *C. trachomatis* infections treated with traditional drugs or to prevent or treat *C. suis* infections caused by tetracycline-resistant strains on pig farms. In particular, in the present study we tested the *in vitro* activity of peptides such as mastoparans, clavanins and polyalanines against *Chlamydia* strains belonging to eight of the eleven known *Chlamydia* species. We selected the *Chlamydia* species pathogens for humans, represented by *C. trachomatis* and *C. pneumoniae*, and the species with a zoonotic potential or causing infections with implications for farming and veterinary industries, such as *C. psittaci*, *C. abortus*, *C. suis* and *C. pecorum*. Additionally, we examined the mouse pneumonia agent *C. muridarum*, widely used to model diseases caused by *C. trachomatis* due to their high relatedness, and finally the recently classified and still little known *C. avium*.

As it is well known, chlamydiae present a unique developmental growth cycle characterized by an infectious elementary body (EB) that enters the cells and, within a cytoplasmic inclusion, is transformed in a non-infectious reticulate body (RB) which multiplies by binary fission for about 21-24 h. At that time, the RBs reorganize into EBs that exit the infected cells 36-48 h post-infection

and invade new cells. In cell monolayers *in vitro*, the number of cells showing cytoplasmic inclusions, containing colonies of chlamydiae, represents the degree of infectivity or infecting forming units (IFU).

In order to determine whether the peptides, including different forms of mastoparans and clavanins,[16, 17] were able to reduce *in vitro* chlamydial infectivity acting directly on *Chlamydia* EBs or on the cells previously infected by *Chlamydia*, EBs purified from the eight *Chlamydia* species, as well as *Chlamydia* infected cells, were treated with the synthetic peptides and then any reduction in chlamydial infectivity was determined. Subsequently, a transmission electron microscopy study was performed on *Chlamydia* infectious EBs that were untreated and on others after their treatment with the active peptides, to better investigate the effects of the peptides on these microorganisms, by detecting possible ultra-structural EBs alterations, such as the appearance of vacuolar degeneration and disrupted membranes.

MATERIAL AND METHODS

Peptide synthesis

The *in vitro* anti-chlamydial activity of the synthetic peptides mastoparan L (ML) (NH_2-INLKALAALAKKIL-COOH) originally, isolated from wasp venom, and its analogue MO[17] (MMO) (under protection), clavanin A (C) (NH_2-VFQFLGKIIHHVGNFVHGFSHVF-COOH)[18] originally purified from the sea tunicate *Styela clava*, and its modified version named clavanin MO (CMO) (NH_2-FLPIIVFQFLGKIIHHVGNFVHGFSHVF-COOH),[16] and *Pa*-MAP 2 (NH_2-LKAAAAAAKLAAKAAKAALKAAAAAAKL-COOH)[19] and 1.9 (NH_2-LAAKLTKAATKLTAALTKLAAALTAAAT-COOH),[20] which are peptides derived from the polyalanine peptide from the Antarctic fish *Pleuronectes americanus*, was investigated. All peptides were synthesized by Peptides 2.0 (Chantilly, VA, USA) by solid-phase with the N-9-

fluorenylmethyloxycarbonyl (Fmoc) technique. Peptides were further purified by high-performance liquid chromatography (HPLC) and the identity of each peptide was confirmed by MALDI-ToF MS (Bruker, Germany) (data not shown). Peptide purity used in biologic assays was > 95%.

Chlamydia strains tested

The strains we tested included 23 human and 13 animal *Chlamydia* strains, belonging to eight different *Chlamydia* species. *C. trachomatis* comprised 18 strains represented by nine isolates from urethral swabs of male patients with non gonococcal urethritis admitted to S. Orsola Hospital (Bologna, Italy) and typed as D, E, F, G, H and K serovars and nine USA reference strains (Ic Cal-8 serovar D, Bour serovar E, Ic Cal-3 serovar F, 392-F serovar G, 580 serovar H, 870 serovar I, UW-36 serovar J, UW-31 serovar K and 434 serovar L2), kindly provided by Prof. Julius Schachter (University of California, San Francisco, USA). *C. pneumoniae* included 5 strains comprising the two reference strains IOL-207 (originally isolated in 1967 in London from an Iranian child with trachoma) and CM-1 (the clinical isolate ATCC VR-1360), provided by the Centers for Disease Control and Prevention, Atlanta, GA, USA, and three Italian strains isolated from patients with pneumonia admitted to S. Orsola Hospital (Bologna). The 13 animal *Chlamydia* strains included: the reference strain Nigg of *C. muridarum*, kindly provided by Dr. Orietta Finco (Novartis, Siena, Italy) the reference avian strain 6BC (USA) of *C. psittaci* (Prof. J. Schachter, University of California, San Francisco, USA), 4 pigeon isolates of *C. psittaci*, 2 *C. abortus* strains isolated from sheep with reproductive disorders, 2 *C. pecorum* isolates from cattle with encephalitis, 1 pigeon isolate of the recently classified *C. avium* species, 2 *C. suis* strains isolated in the Vetswiss Faculty (University of Zurich, Switzerland) from pigs with conjunctivitis. *C. psittaci*, *C. abortus*, *C. pecorum* and *C. avium* strains were isolated in the Diagnostic Section, Zooprophylactic Institute (IZSLER, Pavia, Italy).

In vitro activity of synthetic peptides against *Chlamydia*

Chlamydia strains were cultivated in LLC-MK2 cells[21] (a continuous cell line derived from Rhesus monkey kidney tissue) grown at 37°C, in the presence of 5% CO_2, in 24-well plates containing a glass coverslip (diameter, 12 mm) at the bottom. The cell growth medium was Eagle's minimum essential medium (EMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 10 $mg.l^{-1}$ gentamicin, 10 $mg.l^{-1}$ vancomycin and containing 2mM glutamine and 1.7 $mg.l^{-1}$ glucose. The infectious EBs were purified by use of a sucrose gradient,[22] resuspended in 0.2 M sucrose-phosphate-glutamic acid (SPG), and frozen in 0.1 ml aliquots at -80°C. For each peptide a stock solution of $1g.l^{-1}$ in phosphate-buffered saline, pH 7.4 was prepared and subsequently stored at -80°C. Chlamydial infectivity, or inclusion-forming units (IFU), was determined by counting the number of fluorescent cells with cytoplasmic inclusions that appeared after the cells were infected by chlamydiae. A peptide is considered active if it is able to reduce by ≥50% the number of cells with inclusions in comparison to the cells infected by the same amount of chlamydiae but not treated with the peptide. In order to calculate the lowest peptide concentration capable of prevent the detection of more than 50% of the chlamydial inclusions compared with untreated controls, each peptide was twofold diluted from 80 to 1.25 $\mu g.ml^{-1}$ in phosphate-buffered saline (PBS) pH 7.4 in a volume of 100 µl in polypropylene tubes. Subsequently, 100 µl of 10^3 IFU per ml of purified EBs in SPG were added to an equal volume of each peptide concentration and incubated for 2 h at room temperature. Untreated EBs were used as a control. The 200 µl mixture was added to 800 µl of chlamydial growth medium represented by EMEM supplemented with 10% FCS, glucose ($5mg.l^{-1}$), cycloheximide ($1\ mg.l^{-1}$) and inoculated onto LLC-MK2 cells grown in 24-well plates containing a glass coverslip (diameter, 12 mm) at the bottom. After a centrifugation at 800×g for 1.5 h at 33°C, the plates were incubated at 37°C for 48-72 h in the presence of 5% CO_2, after removing the medium and adding chlamydial growth medium. In parallel, in order to evaluate an activity of peptides on cells infected by chlamydiae, we infected cell monolayers with an inoculum of *Chlamydia* strains that yielded 1×10^2 IFU per ml; after a centrifugation at 800×g for 1.5 h at 33°C, we removed the medium from the infected monolayers and added the synthetic peptides diluted

twofold from 80 to 1.25 $\mu g.ml^{-1}$ in chlamydial growth medium. The cultures were then fixed and stained for the detection of chlamydial inclusions by using a fluorescein-conjugated monoclonal antibody against *Chlamydia* genus-specific antigen (Meridian, Cincinnati, OH, USA). Subsequently, the number of cells containing fluorescent *Chlamydia* inclusions was determined using a Zeiss UV microscope. All tests were run in triplicate and each datum reported is the mean of the data obtained in three repeated experiments.

Transmission electron microscopy analysis

The transmission electron microscopy investigation was performed by using 100 µl of purified *C. pneumoniae* EBs suspended in SPG medium and treated for 2 h at room temperature with 8 µl of peptides (stock solution), in order to achieve a final peptide concentration of 80 $\mu g.ml^{-1}$. The peptide-treated and the untreated EBs were pelleted by ultra-centrifugation for 30 min at 4°C. After removal of the supernatant, the peptide-treated and the untreated EBs were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2-7.4 at 4°C for at least 4 h, washed in 0.1 M cacodylate buffer pH 7.2 and post-fixed in 1% OsO_4 in 0.1 M cacodylate buffer pH 7.2 for 1 h at 4°C. Then, pellets were dehydrated in graded ethanol and embedded in Araldite (Serva). Thin and ultrathin sections were obtained using an ultra-microtome (Ultracut). The ultrathin sections were counterstained with uranyl acetate and lead citrate and examined by a Zeiss EM109 transmission electron microscope.

RESULTS

In comparison with untreated controls, CMO reduced by ≥50% the inclusion numbers of all 36 *Chlamydia* strains at a concentration of 10 $\mu g.ml^{-1}$. In particular, the infectivity reduction of *C. trachomatis*, *C. pneumoniae*, *C. psittaci*, *C. suis*, *C. muridarum*, *C. pecorum*, *C. abortus* and *C. avium* strains tested was greater than 50% after their treatment with CMO at 10 $\mu g.ml^{-1}$ and greater

than 90% at a CMO concentration of 80 μg.ml^{-1} (data not shown). Otherwise, ML at 10 μg.ml^{-1} was able to reduce by ≥50% the infectivity of strains belonging to only four out of the eight *Chlamydia* species tested: *C. trachomatis*, *C. pneumoniae*, *C. suis*, and *C. muridarum*. The cytoplasmic inclusion number of the strains belonging to these *Chlamydia* species was reduced by ≥ 90% at a CMO concentration of 80 μg.ml^{-1} (data not shown). ML did not exert any inhibitory effect on the remaining strains tested, belonging to *C. psittaci*, *C. pecorum*, *C. abortus* and *C. avium* species, even at 80 μg.ml^{-1}. The remaining four out of the six peptides tested (MMO, C, *Pa*-MAP 2 and *Pa*-MAP 1.9) were ineffective against the EBs purified from all 36 *Chlamydia* strains tested, even at 80 μg. ml^{-1} (Table 1). Furthermore, the addition of the six peptides after infecting the cells with EBs of the eight *Chlamydia* species investigated, did not significantly reduce chlamydial infectivity, in comparison to the infected cells not treated with the peptides. In particular, these peptides were not able to reduce by ≥50% the chlamydial IFU, even at the concentration of 80 μg.ml^{-1}. Moreover, CMO and ML peptides, active when incubated with chlamydial EBs before the subsequent cell infection, did not inhibit chlamydial growth when added to already-infected cell monolayers (Table 2).

Interestingly, none of the six peptides showed any cytotoxic effect after LLC-MK2 uninfected cells were treated for 48-72 h with a concentration of each peptide ranging from 80 to 1.25 μg.ml^{-1}. In addition, evident morphological alterations were not visible under the UV microscope in the peptide-treated cells, even at a peptide concentration of 80 μg.ml^{-1}.

The transmission electron microscope investigation on *C. pneumoniae* EBs treated with CMO and ML peptides proved to be active *in vitro* against this *Chlamydia* species, showing a probable membrane vacuolar degeneration particularly evident in CMO-treated EBs in comparison to the integrity and the regular shape of the peptide-untreated EBs. *C. pneumoniae* EBs, treated with ML, showed only occasionally a vacuolar degeneration and membrane alterations (Figure 1). In both cases, peptides lead to the presence of cellular fragments, mainly observed in the left side of Figures

1B and 1C, probably yielded by the presence of antimicrobial peptides utilized. Otherwise, these fragments were not observed in untreated samples (Figure 1A).

DISCUSSION

Chlamydia infections play a major role in both human and animal diseases. In particular, irreversible infertility can result from persistent and unresolved *C. trachomatis* infections, and significant economic and agricultural damage can be caused by *Chlamydia* infections on pig, cattle and poultry farms.[1] The availability of active drugs against *Chlamydia* is therefore useful both for the treatment of human and animal infections and for the prophylactic antibiotic treatment in animal herds.

Although a therapeutic effect on chlamydiae can be expected from tetracyclines, macrolides and quinolones, some cases of human and animal infections with therapeutic failure or infections in swine caused by tetracycline-resistant strains, containing the tetracycline-resistant gene, may occur.[23] Tetracyclines are indeed widely used in veterinary medicine because they are relatively inexpensive and present a broad-spectrum activity.

In this regard, it is important to have molecules available with efficient antimicrobial activity against chlamydiae. In several organisms, antimicrobial peptides, as components of the innate immune system, represent the first barrier against microbial infections and can interact with different targets such as membranes, cell wall, nucleic acid or cytosolic proteins.[24] The mechanism of action of antimicrobial peptides is primarily related to the global positive charge of most of them, leading to interaction with anionic microorganism membranes, leading to the formation of pores, or acting as detergents, among other membrane level mechanisms of action.[12]

With regard to the antimicrobial peptides used in the present study, it is known that CMO interacts with bacterial lipid bilayers resulting in drastic changes in membrane morphology,[25] Mastoparan

analogues (with an extra positive charge at the C-terminal) cause disruption of bacterial membrane[12] and *Pa*-MAP antimicrobial activity can be attributed mostly to its hydrophobic interactions.[14] CMO has demonstrated antimicrobial activity against *S. aureus* and *K. pneumoniae*,[25] with mastoparan analogues working against *Acinetobacter baumannii*[12] and *Pa*-MAP against *S. aureus* and *E. coli*.[26]

As concerns the *in vitro* activity of synthetic peptides against *Chlamydia* spp., our previous study demonstrated the ability of the cathelicidin SMAP-29 to reduce by ≥ 50% the inclusion numbers of all the *C. trachomatis* and *C. pneumoniae* strains tested at 10 μg ml^{-1}, while none of the animal *Chlamydia* strains tested were sensitive to SMAP-29[10].

In the present study, two out of the six peptides that were tested *in vitro* proved to be active against chlamydiae. CMO demonstrated a high and broad spectrum activity since the peptide, in contrast with the original clavanin A at the concentration of 10 $\mu g.ml^{-1}$, reduced by ≥50% the infectivity of all strains tested, belonging to eight *Chlamydia* species, including the new and recently identified *C. avium* species. Otherwise, the eight *Chlamydia* species tested showed different susceptibility to ML, with *C. trachomatis*, *C. pneumoniae*, *C. muridarum*, and *C. suis* being susceptible at 10 $\mu g.ml^{-1}$ and *C. psittaci*, *C. pecorum*, *C. abortus*, *C. avium* not sensitive to this peptide, even at 80 $\mu g.ml^{-1}$. Similar data were observed by Silva *et al.*,[16] in a study that showed the improvement of activity caused by addition of a short tag, engineered at the N-terminus of original clavanin. Moreover, similar effects were obtained with another modification added to ML (unpublished data). Last but not least, the two polyalanine peptides *Pa*-MAP 2 and *Pa*-MAP 1.9 here studied were, surprisingly, not active against *Chlamydia*, as observed against other bacterial pathogens.[19, 20]

The inhibitory activity showed by ML against only a few chlamydial species tested, in comparison to CMO, which is active against all the *Chlamydia* species investigated, suggests that variations in the chlamydial membrane composition can influence the susceptibility to the peptides.

It is noteworthy that the anti-chlamydial activities of CMO and ML were not related to their ability to enter *Chlamydia*-infected cells and subsequently to inhibit intracellular chlamydial growth, but were due instead to a specific activity directed against infectious chlamydial forms.

Our observations by transmission electron microscopy analysis suggest that CMO can act at the level of the bacterial membrane, since disrupted membranes were observed in *Chlamydia* EBs treated with the peptide. These observations are in accordance with the finding that clavanins and mastoparans interact with lipid bilayers, resulting in drastic changes in membrane morphology, previously reported in literature.[27,28] Another interesting point is that clavanins do not shown a typical membrane disturbance activity. This was observed by Silva *et al*.[18], which showed that intra-cellular effects could also be detected, suggesting multiple targets. Similar data was also here observed. In Figure 1B and C it is clearly observed that several cells did not present membrane disturbance, indication that not only membranes are affected by peptides here utilized. Furthermore, the activity of CMO we observed on *Chlamydia* EBs in the transmission electron microscopy analysis is similar to that showed in our previous ultra-structural study on *Chlamydia* EBs treated with the cathelicidin SMAP-29, causing the loss of integrity of most particles[9].

However, in this respect we can point out that, although cathelicidins and other host defense peptides have been shown to be effective in some *in vivo* studies, their effective dose is very close to the toxic dose.[29] In addition, they are cytotoxic to mammalian cells. The lack of CMO cytotoxicity highlighted in our investigation could be similar to the findings of a previous study where clavanin A showed no cytotoxic activity against mammalian cells and in acute toxicity tests[13].

The transmission electron microscopy analysis suggests also for ML a permeabilization effect on the *Chlamydia* EB membrane, even though it is less pronounced compared to that of CMO: this observation is in agreement with the well-known ability of mastoparan to interact with the phospholipid constituents of animal and bacterial cell membranes.[30]

The availability of active anti-chlamydial peptides could be very useful if we focus on some observations: the reported tetracycline treatment failure in some *C. trachomatis* human infections, the *in vitro* evidence of the adaptability of *C. trachomatis* to evolve to antibiotic resistance and the widespread prevalence of *C. suis* tetracycline-resistant strains on pig farms. In addition, the impact on human and animal health of the selection for antimicrobial-resistant *Chlamydia* strains in the animal reservoir is not sufficiently known. Further studies are needed to consider the peptides we tested in the present study as potential and promising compounds in the therapy of chlamydial infections with treatment failure. Furthermore, if these peptides will be active *in vivo* at the concentrations that have been shown to be active *in vitro*, in the absence of toxic effects, they could be used in humans as well as in animals to prevent chlamydial infections.

CONCLUSION

We showed a high and specific *in vitro* activity of the two synthetic peptides CMO and ML, belonging to clavanins and mastoparans, respectively, against the infectious forms (EBs) of some *Chlamydia* species relevant in human and animal diseases, in the absence of toxic effect against mammalian cells. We highlighted also a broader anti-chlamydial activity of CMO in comparison to ML.

The transmission electron microscopy analysis showed a loss of integrity of the peptide-treated EBs, particularly evident in CMO-treated EBs.

These findings, combined with the already proven *in vitro* and *in vivo* activity of CMO and ML against both Gram-positive and Gram-negative bacteria, suggest that these peptides and primarily CMO could be attractive candidates for the treatment of chlamydial unresolved or persistent human infections as well as for the prevention and treatment of chlamydial long-term infections on livestock farms, particularly pig farms where tetracycline resistant *C. suis* strains circulate.

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Author contributions

Conceived and designed the experiments: MD, GC, RB, VP, NB, SM, OF

Performed the experiments: MD, GC, RB, VP, EVN, AL, OF

Analyzed the data: MD, GC, RB, VP, EVN, GAP, AL, OF

Contributed reagents/materials/analysis tools: MD, GC, VP, NB, SM, OF

Wrote the paper: MD, GC, VP, OF

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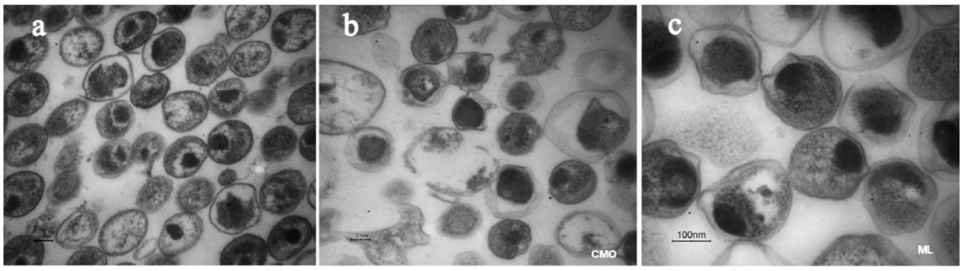


Figure 1 - Transmission electron micrographs of Chlamydia pneumoniae EBs. (a) Untreated EBs; (b) EBs treated with CMO; (c) EBs treated with ML. 185 × 52mm (300 × 300 DPI)

124x35mm (300 x 300 DPI)

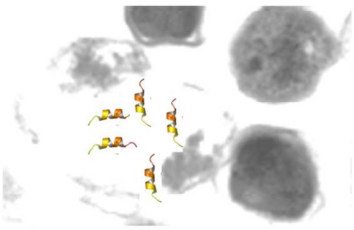
Table 1. Activity of clavanins (CMO and C), mastoparans (ML and MMO), and polyalanins *Pa*-Map 2 and 1.9 peptides against EBs of different *Chlamydia* spp.

	Peptide concentration (μg.ml^{-1}) reducing chlamydial inclusions by ≥50%					
Strains (n)	**CMO**	**C**	**ML**	**MMO**	***Pa*-Map 2**	***Pa*-Map 1.9**
***C. trachomatis* (18)**	10	>80	10	>80	>80	>80
***C. pneumoniae* (5)**	10	>80	10	>80	>80	>80
***C. psittaci* (5)**	10	>80	>80	>80	>80	>80
***C. suis* (2)**	10	>80	10	>80	>80	>80
***C. muridarum* (1)**	10	>80	10	>80	>80	>80
***C. pecorum* (2)**	10	>80	>80	>80	>80	>80
***C. abortus* (2)**	10	>80	>80	>80	>80	>80
***C. avium* (1)**	10	>80	>80	>80	>80	>80

Table 2. Activity of clavanins (CMO and C), mastoparans (ML and MMO), and polyalanins *Pa*-Map 2 and 1.9 peptides against cells infected with EBs of different *Chlamydia* spp.

	Peptide concentration (μg.ml^{-1}) reducing chlamydial inclusions by ≥50%					
Strains (n)	**CMO**	**C**	**ML**	**MMO**	***Pa*-Map 2**	***Pa*-Map 1.9**
***C. trachomatis* (18)**	>80	>80	>80	>80	>80	>80
***C. pneumoniae* (5)**	>80	>80	>80	>80	>80	>80
***C. psittaci* (5)**	>80	>80	>80	>80	>80	>80
***C. suis* (2)**	>80	>80	>80	>80	>80	>80
***C. muridarum* (1)**	>80	>80	>80	>80	>80	>80
***C. pecorum* (2)**	>80	>80	>80	>80	>80	>80
***C. abortus* (2)**	>80	>80	>80	>80	>80	>80
***C. avium* (1)**	>80	>80	>80	>80	>80	>80

Activity of synthetic peptides against *Chlamydia*



338x190mm (96 x 96 DPI)